Sources and pathways for pharmaceuticals in the urban water environment

Rebecca Tuckwell, Michael Revitt, Hemda Garelick, Huw Jones and Bryan Ellis

Urban Pollution Research Centre, Middlesex University, The Burroughs, Hendon, London NW4 4BT, UK
**Corresponding author, e-mail m.revitt@mdx.ac.uk*

ABSTRACT
The progress of five pharmaceutical compounds (bezafibrate, carbamazepine, diclofenac, ibuprofen and sulfasalazine) and one antibacterial agent (triclosan) were monitored through the treatment stages of a large sewage treatment works (STW) using activated sludge as well as in the receiving water both upstream and downstream of the effluent discharge. All except sulfasalazine were detected in the influent at concentrations ranging from 1.44-3.75 μg/L. The analysis of prescription data has been used as a tool to predict the amount of pharmaceuticals potentially released into the catchment of the investigated sewage treatment works and the results compared with the measured influent concentrations. A reduction in concentration between influent and final effluent samples (51-97%) indicates the variable removal of these compounds and therefore their potential to be discharged into receiving surface waters. The analysis of primary and final effluents highlight the important processes involved in the removal of pharmaceuticals and indicate that sorption processes are important for bezafibrate, carbamazepine and diclofenac. These three PPCPs were observed at higher concentrations (0.07-0.35 μg/L) downstream of the discharged effluent compared to upstream (0.02-0.04 μg/L) although the risks that these compounds pose in the environment are not yet fully understood.

KEYWORDS
Pharmaceuticals; prescription data; receiving waters; sewage treatment works.

INTRODUCTION
The quality of natural waters is under threat from the chemical substances discharged in industrial and domestic wastes. As a consequence threshold water quality standards are enforced under legislation (e.g. The Water Framework Directive (2000/60/EC) (Defra, 2009) and Clean Water Act Section 402 (US Environmental Protection Agency, 2009)). Currently legislation is focused on reducing 'priority pollutants' which include a wide range of persistent organic compounds and heavy metals. However, new emerging pollutants which are gaining increasing attention include pharmaceutical compounds and the active ingredients used in personal care products (collectively termed PPCPs) (Ternes, 1998). Through processes such as excretion or disposal of unused or expired drugs, many pharmaceuticals and their metabolites find their way to sewage treatment plants where they are rarely completely eliminated. This results in their continuous release into the aquatic environment through the discharge of final treated effluents. PPCPs also accumulate in sewage sludges and ultimately can be released into the environment through the application of the sludge as an agricultural fertilizer.

There are increasing numbers of publications reporting the detection of trace levels of PPCPs in the influents to sewage treatment works (STW) (Karthikeyan and Meyer, 2006; Zorita *et. al.*, 2009), the effluents from STWs (Clara *et. al*., 2005) and in river waters (Gros *et. al*., 2006 and 2007) at typical concentrations of nanogram per litre to low microgram per litre. Although these low concentrations may not have a therapeutic effect on humans, the potential affect on aquatic ecosystems is still relatively unclear. Despite the lack of full impact related data, the use of these compounds will continue to increase with increasing population size and demand and they will ultimately end up in natural waters. Prescribed pharmaceuticals in human medicine alone have risen in cost in the USA from $433 billion in 2002 to $808 billion in 2009 (IMS Health Market Prognosis, 2009) and new pharmaceutically active substances are continually being developed and introduced into the market place. This paper examines the passage of 6 PPCPs (carbamazepine, sulfasalazine, bezafibrate, diclofenac, triclosan and ibuprofen) through a large sewage treatment plant and assesses the impact of the treated effluent on the receiving water by comparing the pollutant concentrations both upstream and downstream of the discharged effluent.

METHODOLOGY

Sample location and collection

Wastewater samples were collected from a large sewage treatment works (STW) in London at four points through the treatment process (influent, primary effluent, secondary effluent and final effluent). Surface water samples were collected both upstream and downstream of the effluent discharge. The STW receives approximately 244,000 m^3 per day from a 399 km^2 catchment serving a total population of 870,000. The works applies primary sedimentation, followed by secondary activated sludge (13 h) before discharge. All samples were collected in clean amber 2.5 L bottles, filtered on the day of collection and stored at 4°C until extraction (within 4 days).

Chemicals and reagents

Methanol, acetonitrile, formic acid and ammonium acetate were purchased from Fisher Scientific UK Ltd (Leicestershire, UK) and were either HPLC or LCMS grade. Pharmaceutical standards (purity ≥ 95% HPLC) of bezafibrate, carbamazepine, diclofenac, ibuprofen, sulfasalazine and triclosan were purchased from Sigma-Aldrich (Dorset, UK). Stock solutions (200 µg/mL) of all the analytes were prepared in LCMS grade methanol and stored at -80°C. Spiking solutions and external standards were prepared from the stock solution and diluted with 5% (v/v) methanol in LCMS grade water on the day of extraction and stored at 4°C. Samples were filtered with glass microfiber filters (1.2 µm) from Whatman Ltd, UK and extracted with 500mg/6 mL Strata-X cartridges purchased from Phenomenex, UK.

Sample extraction

Samples were divided into 6 equal aliquots (100 mL for the influent, 200 mL for the effluent and 1000 mL for the surface water) and spiked at varying concentrations with the spiking solution before extraction. Strata-X cartridges were first conditioned with 6 mL methanol and equilibrated with 6 mL LCMS grade water before samples were percolated through at an approximate flow rate of 1-2 mL minute using a vacuum extraction manifold (Phenomenex, UK). The sorbent was washed with 6 mL water and dried under vacuum for at least 30 minutes prior to extraction with methanol (10 mL). The resulting extract was evaporated

under a gentle nitrogen stream using a TurboVap (Biotage, Sweden) at 35°C and reconstituted with 0.2 mL 5% (v/v) methanol in LCMS grade water, before transferring to 0.2 µm nylon Mini-UniPrep filter vials (Whatman Ltd) for analysis.

Analysis

Analysis of the extracts was performed with reverse phase high performance liquid chromatography mass spectrometry LC-MS^n with electrospray ionization in positive ($^+$ve) and negative ($^-$ve) ionization modes (LC2010, Shimadzu).

The PPCPs were separated with a Kinetex 2.1 mm x 50 mm C18 column (Phenomenex, UK). For those compounds (bezafibrate, carbamazepine, diclofenac and sulfasalazine) analysed with positive ($^+$ve) ionization, a mobile phase of water (A) and acetonitrile (B) with 0.1% formic acid was used. The solvent gradient started at 5% B and reached 67% B in 20 mins before increasing to 95% B for 5 mins and then returning to 5% B for 10 mins. For ibuprofen and triclosan which were analysed using negative ($^-$ve) ionisation, a mobile phase of water (A) and acetonitrile (B) with 10 mM ammonium acetate was used. A solvent gradient increasing from 5 to 50% B was applied during 15 mins followed by 100% B for 5 mins and then column re-equilibration at 5% B for 10 mins. The column was maintained at 30°C with a flow rate of 0.2 mL/min and an injection volume of 10 µL.

Method validation

The target compounds were monitored using their parent ion in selective ion monitoring (SIM) mode. In positive mode, the following single parent ions $[M+H]^+$ were monitored: bezafibrate: 362, carbamazepine: 237, diclofenac: 296 and sulfasalazine: 399. In negative mode, ibuprofen and triclosan were monitored with parent ions $[M+H]^-$ at 205 and 287, respectively. Quantification of the compounds of interest was performed using the standard addition method. Different concentrations were spiked into separate aliquots of each sample. The real analyte concentration was then determined by linear regression. At least five concentration points were used to check the linear range of the method and r^2 values higher than 0.95 were obtained. To evaluate the method reproducibility (precision) for the individual compounds, surface water samples and effluents were spiked at 100 and 600 ng/L, respectively and divided into aliquots for separate extraction and analysis. It was difficult to determine the LOQ for effluent and surface waters, as the samples already contained the compounds of interest. Therefore, LOQs were estimated from different samples using signal-to-noise ratios of 10. The method validation data is presented in Table 1.

Table 1. The linear range, method precision (%RSD) and limit of quantification (LOQ) determined for the selected PPCPs in sewage effluent and river water.

Drug	r^2 for linear range (5 point calibration)		Precision (RSD %) (n=3)		LOQ (ng/L)	
	Effluent	River water	Effluent	River water	Effluent	River water
Bezafibrate	0.9547	0.9983	3.4	2.7	100	5
Carbamazepine	0.9838	0.9787	6.0	2.5	100	5
Diclofenac	0.9813	0.9975	13.0	1.9	150	19
Ibuprofen	0.9885	0.9968	21.0	9.0	242	68
Sulfasalazine	0.9670	0.9634	54.2	40.9	150	65
Triclosan	0.9949	0.9752	17.4	11.0	250	80

n= number of samples. LOQ was estimated for each sample matrix (effluent or river water) at a signal to noise ratio 10.

RESULTS AND DISCUSSION

The concentrations of the six selected PPCPs detected in the influent, effluents and surface water samples are presented in Figure 1. Complete data sets have been obtained for carbamazepine, bezafibrate and diclofenac. The influent concentrations for sulfasalazine could not be reliably determined due to the poor extraction efficiency from raw sewage. For triclosan and ibuprofen, only the raw and settled sewage samples demonstrated concentrations which were clearly above the levels of quantification. The upstream concentrations plotted in Figure 1 have been increased by a factor of 10 for clarity.

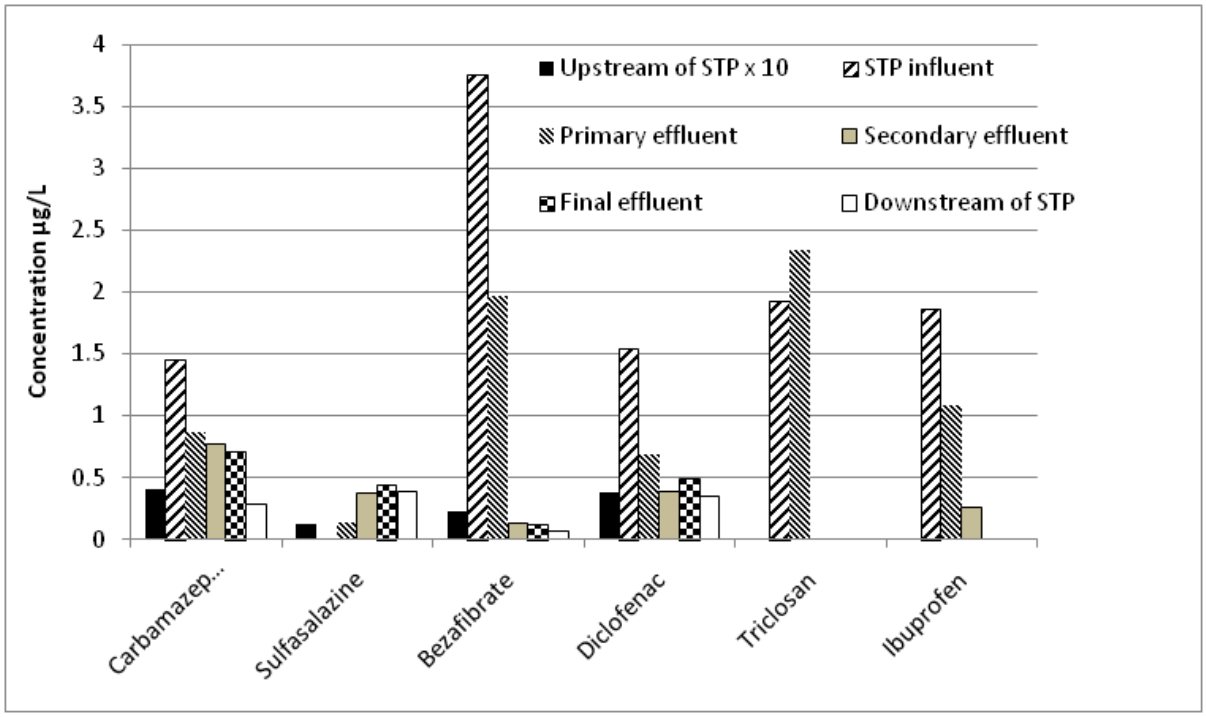

Figure 1. Concentrations of selected PPCPs in the influent and effluents of a sewage treatment plant and in the receiving water upstream and downstream of the final effluent release point.

Comparison of predicted concentrations and measured concentrations

The range of monitored sewage treatment influent concentrations (1.44 µg/L for carbamazepine to 3.75 µg/L for bezafibrate) is consistent with previously reported values (Clara *et. al.*, 2005). Several factors can influence these concentrations including consumption levels, population characteristics and the age and design of the sewer system. Consumption data are considered to be a critical influencing parameter but this information can be difficult to locate. For example, in England it is difficult to obtain a reliable estimate of the tonnes of pharmaceuticals used per year and currently a central or regional record of pharmaceutical use by hospitals or over-the-counter medicines is not readily accessible making it difficult to quantify the amount of pharmaceuticals entering the environment. However, prescription analysis and cost (PACT) data is collated from all the prescriptions dispensed in the community (community pharmacists and dispensing doctors) in England (NHS Information Centre, 2009) and is readily available. Using PACT data, the quantities of

Table 2. Prescription data for selected PPCPs in England for 2007 and estimated influent concentrations to a sewage treatment works.

PPCP	Class	Tonnes/year (2007)	% excreted unchanged	Predicted maximum concentration (µg/L)
Bezafibrate	Lipid regulating drug	10	50	~2.0
Carbamazepine	Anticonvulsant	55	<10	~10.0
Diclofenac	Non-steroidal anti-inflammatory	30 +	15	~6.0
Ibuprofen	Non-steroidal anti-inflammatory	108 +	<10	~20.0
Sulfasalazine	Sulfanilamide	49	15	~9.20
Triclosan	Antibacterial agent	++	n/a	n/a

+ Available without prescription, ++ active present in numerous cosmetic products, n/a not available.

bezafibrate, carbamazepine, diclofenac, ibuprofen and sulfasalazine used per annum have been estimated (Table 2) and shown to vary between 10 and 108 tonnes/year in 2007, indicating that there are significant differences in the types and amounts of pharmaceuticals consumed. By scaling down these estimates to the sewage treatment works catchment containing 870,000 population equivalent and taking into account the typical dry weather flow of 244,000 m^3/day, a predicted maximum concentration entering the STW has been estimated (Table 2). The observed influent concentrations for carbamazepine and ibuprofen (1.44 µg/L and 1.85 µg/L) are consistent with the predicted maximum concentration for the catchment area (~10 and 20 µg/L) when the fact that <10 % of these compounds are excreted unchanged is taken into account. However, ibuprofen is also available without prescription and therefore higher influent concentrations could be expected.

The predicted maximum concentration for diclofenac (~6.0 µg/L) was higher than measured in the influent concentration (1.54 µg/L) but this would reduce to less than 1 µg/L when the percentage excreted is taken into account, The reverse is observed for bezafibrate where the predicted concentration (~2.0 µg/L) is lower than measured in the influent (3.75 µg/L) and this difference would be magnified when the percentage of bezafibrate excreted unchanged (50%) is taken into account. A predicted maximum concentration for triclosan was not estimated as this compound is present as an active component in many cosmetic and cleaning products and is therefore accessible without prescription. It is also generally applied externally rather than being ingested. If over the counter drugs sales and regional and seasonal pharmaceutical usage data was available a more accurate prediction could be made.

Reduction of PPCPs through STW processes

Where the relevant monitoring date is available, the general trend observed is a decrease in PPCP concentrations through the sewage treatment process with overall reductions of 97%, 84%, 69% and 51% for bezafibrate, ibuprofen, diclofenac and carbamazepine, respectively between raw sewage and the final treated effluent (secondary effluent for ibuprofen) (Figure 1). The removal of PPCPs from wastewaters is a complex process but two particularly important mechanisms are sorption and biodegradation at the primary and secondary treatment stages, respectively. Carbamazepine was only reduced by 11% during activated sludge treatment (Figure 1) indicating that it is not easily biodegraded under the applied conditions. In contrast, 40% of carbamazepine was removed by primary sedimentation suggesting that sorption is a more important removal mechanism. This is consistent with both the soil organic carbon-water partitioning coefficient (K_{oc}) for carbamazepine (510) and the sludge-water adsorption coefficient (K_d) (25 mL/g) which are indicative of a moderate level

of adsorption to activated sludge. It has been reported that carbamazepine is not removed during the sewage treatment process (Radjenovic *et. al*., 2007) but this clearly varies between treatment plants due to influencing factors being operating conditions, age of sludge and treatment plant design.

In the removal of bezafibrate both primary and secondary sewage treatment processes contributed to the high removal rate (97 %) and this is consistent with removal values reported elsewhere (Castiglioni *et.al*., 2004). Sedimentation reduced the bezafibrate concentration by 48% and a similar decrease was observed during activated sludge treatment. Primary sedimentation processes were also important in the removal of diclofenac (57%) which is consistent with the adsorption potential to suspended solids and sediment predicted by a K_{oc} value of 830. The total removal percentage (69%) observed for diclofenac throughout the treatment process agrees with the results reported by Roberts and Thomas (2006) although the slight increase in diclofenac concentration between secondary and final effluent is unexpected and contrary to previous (Kasprzyk-Hordern *et.al.*, 2009).

The incomplete removal of PPCP compounds in the sewage treatment process will pose problems for the receiving waters as evidenced by the consistently increasing downstream levels. The limited removal of bezafibrate, carbamazepine and diclofenac resulted in downstream concentrations of 0.07, 0.28 and 0.35 µg/L respectively compared to 0.02, 0.04 and 0.04 µg/L upstream.

CONCLUSIONS

The analysis of prescription data has indicated the high quantities of 6 PPCPs (bezafibrate, carbamazepine, diclofenac, ibuprofen, sulfasalazine and triclosan) prescribed per year that could ultimately arrive at sewage treatment plants following ingestion and excretion. The analysis of influents, effluents and samples collected both upstream and downstream of the effluent discharge from a large sewage plant show that these compounds are incompletely eliminated. Although the percentage removed during sewage treatment depends on a number of factors including the type of treatment and the population characteristics, sorption is shown to be an important removal process. A comparison of PPCP concentrations upstream and downstream of the effluent discharge suggests the potential for pharmaceuticals to accumulate in receiving waters.

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